

LMW-HA. Therefore, chondrocyte MyD88 expression is critical for coupling inflammation and innate immunity with the potential progression of OA. Chondrocyte MyD88 function represents a novel potential therapeutic target in OA.

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BONE MARROW LESIONS FROM OSTEOARTHRITIS KNEES ARE CHARACTERIZED BY SCLEROTIC BONE THAT IS LESS WELL MINERALIZED

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Purpose: Although the presence of bone marrow lesions (BML) on MRI is strongly associated with osteoarthritis (OA) progression and pain, the underlying pathology is not well established. The lack of knowledge on what contributes to these lesions hampers their clinical utility. The aim of this study was to evaluate the architecture of subchondral bone in regions with and without BMLs from the same individual using bone histomorphometry.

Methods: Postmenopausal female subjects with predominantly medial compartment OA, on a waiting list for total knee replacement (TKR) were recruited. To identify the location of the BMLs, subjects had an MRI performed on their study knee prior to TKR using a Philips 3.0T scanner with a dedicated extremity coil. The following imaging sequence was used on each patient: Sagittal dual echo FSE fat suppressed/WE with TR/Te of ~4000ms/15ms, 60ms, 2.5- 3mm slices, no skip/gap, 256x256 matrix, 12cm FOV (for distal femur and proximal tibia). An axial map of the tibial plateau was made delineating the precise location of the BML. After surgical removal of the tibial plateau, the BML was localized using the axial map from the MR image and the lesion excised along with a comparably sized bone specimen adjacent to the BML and from the contralateral compartment without a BML. Cores were taken from the excised material and the mineral density, bone volume fraction (BV/TV), and trabecular number (Tb.N), thickness (Tb.T), and spacing (Tb.Sp) of the cortical-like subchondral plate and the subchondral trabecular bone were

assessed using micro-computed tomography imaging (Scanco CT40). We computed the mean and SD for each parameter, and the unaffected bone from the medial tibial plateau and the bone from the lateral tibial plateau were compared with the affected BML region in the medial tibial plateau.

Results: 5 participants ranging in age from 48 to 90 years of age were recruited. The table depicts the micro-CT parameters from the affected (medial), unaffected medial and unaffected lateral compartments of the knee.

A representative image from the medial tibial plateau affected by BML, medial tibial plateau unaffected by BML and the lateral tibial plateau unaffected by BML is presented; the sub-chondral plate is orientated at the top.

Conclusions: Our preliminary data localizes specific changes in bone mineralization, remodeling and defects within BML features that are adjacent to the sub-chondral plate. These BMLs appear to be sclerotic compared to unaffected regions from the same individual based on the increased bone volume fraction and increased number of trabeculae. However, the mineral density in these lesions is reduced and may render this area mechanically compromised, and thus susceptible to attrition. Further work is required to evaluate whether these observations are caused by an increase in synthesis or a decrease in resorption of the bone.

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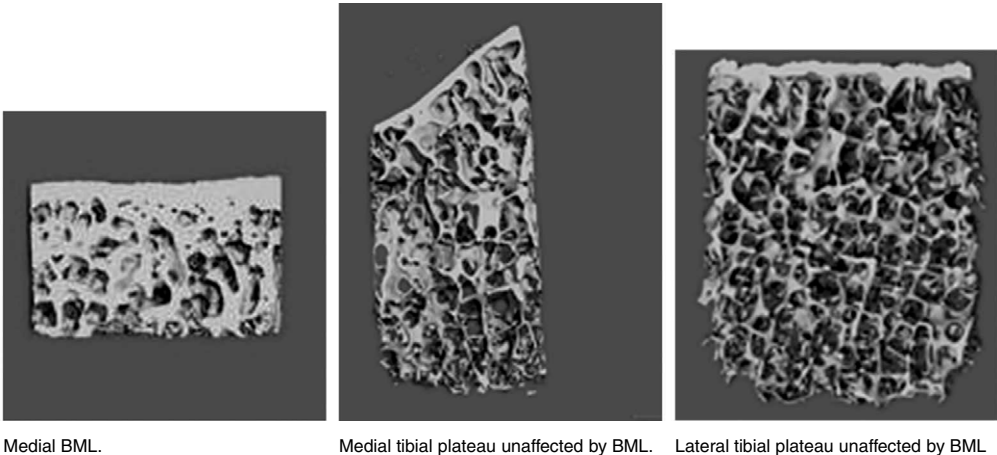
THE REMODELLING PROCESS OF THE SUBCHONDRAL BONE DURING HUMAN OSTEOARTHRITIS IS CLOSELY DEPENDENT ON RANKL MODULATION

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Purpose: Osteoarthritis (OA) is the most common joint pathology and recent studies have suggested that the subchondral bone metabolism is intimately involved in cartilage degradation and loss. Bone remodelling is tightly regulated by a molecular triad composed of OPG/RANK/RANKL. RANKL enhances osteo-

Abstract 12 – Table 1

| | Medial tibia affected by BML | Medial tibia unaffected by BML | p value comparing medial BML with unaffected medial plateau | Lateral tibial plateau | p value comparing medial BML with lateral tibial plateau |
|-----------------------------|---------------------------------|-----------------------------------|--|---------------------------|---|
| BV/TV | 0.27 (0.07) | 0.16 (0.03) | 0.01 | 0.16 (0.06) | 0.03 |
| Mineral density (mg HA/ccm) | 1037.97 (23.31) | 1089.27 (21.38) | 0.007 | 1050.94 (18.86) | 0.36 |
| Tb.N (1/mm) | 2.74 (0.38) | 1.20 (0.32) | 0.0001 | 2.56 (0.87) | 0.68 |
| Tb.Th (mm) | 0.31 (0.09) | 0.23 (0.02) | 0.09 | 0.23 (0.07) | 0.15 |
| Tb.Sp (mm) | 0.38 (0.13) | 1.01 (0.32) | 0.004 | 0.45 (0.23) | 0.57 |



clastogenesis via interaction with its receptor, RANK, whereas OPG inhibits this osteoclastogenesis by acting as a soluble RANKL receptor competitor. We previously reported that human OA subchondral bone osteoblasts could be discriminated into two subpopulations identified by their levels of endogenous production (low [L] or high [H]) of PGE₂. Firstly, we analysed and compared the histology of human subchondral bone from normal, L and H OA. Then, we investigated on each of these human subchondral bone osteoblast categories the OPG and RANKL levels on these two factors, as well as the activity of each OA osteoblast category on the osteoclast differentiation. Further, we determined on L and H OA osteoblasts the modulation of some bone remodelling factors on the RANKL level.

Methods: Histology of the human subchondral bone was performed on knee specimens. Gene expression was determined using real-time PCR, PGE₂ and OPG protein production by specific ELISA, and membranous RANKL by flow cytometry methodology. Osteoclast differentiation and formation was assayed by using the pre-osteoclastic cell line RAW 264.7, which differentiates only in the presence of RANKL. Modulation of membranous RANKL on L and H OA osteoblasts was monitored following treatment with osteotropic factors.

Results: Histology analysis revealed that the L OA osteoblasts had a reduced subchondral bone mass compared to normal, in contrast to the H OA which showed an increase. The OPG/RANKL mRNA ratio was significantly decreased in L OA compared to normal or H OA, and markedly increased in H OA compared to normal, suggesting an increased level of remodelling activity in L OA cells. This was further supported by the membranous RANKL levels which were significantly elevated in L OA compared to the H OA and normal cells. Moreover, L OA, but not H OA, cells induced a significantly higher level of osteoclast differentiation and formation. Experiments with osteotropic factors revealed that in L OA osteoblasts, TNF- α and Vitamin D₃ significantly increased the membranous RANKL level, a situation not found for the H OA osteoblasts.

Conclusions: The differential abnormal levels of OPG and RANKL, and consequently the OPG/RANKL ratio in the two human OA subchondral bone osteoblast subpopulations, indicate different stages of this diseased tissue, in which the L OA osteoblasts favour bone resorption/remodelling activity. This appears to be related to an increased level of RANKL. A better understanding of the modulation of subchondral bone RANKL during the OA process should aid the development of a specific therapeutic.

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PROTEINASE-ACTIVATED RECEPTOR (PAR)-2 IN OSTEOARTHRITIC SUBCHONDRAL BONE OSTEOBLASTS: IDENTIFICATION OF A NEW THERAPEUTIC TARGET

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Purpose: Proteinase-activated receptors (PARs) represent a family of 7-transmembrane domain G protein coupled receptors. PARs are activated by a unique mechanism of cleavage generating a tethered activating ligand at the N-terminus. One of the members of this family, PAR-2, has been shown to be involved in inflammatory pathways. Our recent work demonstrated that PAR-2 plays an important role in catabolic and proinflammatory pathways in cartilage degradation during osteoarthritis (OA) in humans. Studies have also shown that subchondral bone alteration/remodelling is intimately involved with cartilage

degeneration. Bone remodelling is closely regulated by a molecular triad composed of OPG/RANK/RANKL, in which OPG and RANKL are produced by osteoblasts. RANKL is known to be an important factor in the induction of osteoclastogenesis, whereas OPG inhibits this process. The aim of this study was to evaluate the involvement of PAR-2 in human OA subchondral bone. To this end, we investigated on human subchondral bone osteoblasts the levels of PAR-2, OPG, and RANKL, comparing normal with OA. The role of PAR-2 on the major bone remodelling factors, OPG and RANKL, was also evaluated on OA subchondral bone osteoblasts.

Methods: The in situ levels of PAR-2, OPG and RANKL expression were determined using real time RT-PCR. The level of PAR-2 protein was analysed by Western blot, the membranous RANKL by flow cytometry and OPG by a specific ELISA.

Results: PAR-2 was expressed and produced in human normal subchondral bone osteoblasts, and a statistically significant ($p < 0.05$) increase found in the OA cells. Both RANKL and OPG expression were increased in OA subchondral bone osteoblasts, as were the RANKL membranous protein levels and OPG production ($p < 0.03$, $p < 0.05$, respectively). PAR-2 activation with a specific agonist, SLIGKV-NH₂ (PAR-2-AP; 400 μ M), resulted in a significant increase ($p < 0.0001$) in membranous RANKL on both normal and OA osteoblasts, but had no effect on OPG production, indicating that PAR-2 activation favours bone resorption.

Conclusions: This study demonstrates an increased level of PAR-2 in human OA subchondral bone osteoblasts, and shows, for the first time, that specific PAR-2 activation upregulates a major factor (RANKL) involved in the remodelling process of this tissue. These results shed new light on the involvement of PAR-2 in OA pathophysiology, suggesting PAR-2 as a potential new therapeutic approach for the treatment of OA, not only for cartilage but also at the subchondral bone level

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EP2/EP4 MEDIATED PGE2 SIGNALLING INDUCES THE SYNTHESIS OF OSTEOPROTEGERIN (OPG) AND RECEPTOR ACTIVATOR OF NF-KAPPA B LIGAND (RANKL) IN HUMAN OSTEOARTHRITIC CHONDROCYTES IN CULTURE

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Purpose: Cartilage degeneration is the central feature in osteoarthritis (OA), but it is associated with concomitant changes in all the structures of the joint, in particular the subchondral bone. Articular chondrocytes express and synthesize OPG, RANK and RANKL. However, in spite of the relevance of these proteins in the adjacent bone metabolism, there is still little known about their regulation in the cartilage. Our aim was to explore the role of PGE₂, the eicosanoid found at the higher concentration in OA joints, in the expression and synthesis of OPG and RANKL in human osteoarthritic chondrocytes (HOC) in culture.

Methods: HOC were obtained from the joint specimens of OA patients who underwent total knee replacement surgery. The gene expression of OPG and RANKL were assessed in HOC stimulated with PGE₂ by quantitative PCR, and the corresponding protein synthesis was studied by western-blot. We also examined which of the four different PGE₂ receptors (EP receptors) was involved in the PGE₂ action using specific EP agonists.

Results: PGE₂ elicited a dose and time-dependent increase in the gene expression and protein synthesis of OPG, with a peak at 24 hours of incubation (gene expression: 2.8-fold increase; protein: 3.5-fold increase, both for 10-6M PGE₂ vs. unstimulated HOC). Exposure to PGE₂ also resulted in a dose and time-dependent increase in the presence of RANKL, peaking at 24 h of incubation (gene expression: 8-fold for 10-6M PGE₂ vs. un-